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Research article

Intrathecal insulin-like growth factor 1 but not insulin enhances myelin repair in young and aged rats

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HIGHLIGHTS

- Insulin-like growth factor 1 (IGF-1) enhanced remyelination in young and aged rats.
- Insulin, functionally related to IGF-1, did not enhance remyelination.
- Aged rats show less pronounced myelin repair compared to young rats.
- Aged rats are model for chronic demyelination faced in chronic multiple sclerosis.

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ABSTRACT

One main pathological hallmark of multiple sclerosis (MS) is demyelination. Novel therapies which enhance myelin repair are urgently needed. Insulin and insulin-like growth factor 1 (IGF-1) have strong functional relationships. Here, we addressed the potential capacity of IGF-1 and insulin to enhance remyelination in an animal demyelination model *in vivo*. We found that chronic intrathecal infusion of IGF-1 enhanced remyelination after lysolecithin-induced demyelination in the spinal cord of young and aged rats. Aged rats showed a weaker innate remyelination capacity and are therefore a good model for progressive MS which is defined by chronic demyelination. In contrast to IGF-1, Insulin had no effect on remyelination in either age group. Our findings highlight the potential use of IGF-1 as remyelinating therapy for MS, particularly the progressive stage in which chronic demyelination is the hallmark.

1. Introduction

Multiple sclerosis (MS) is a disease with multifactorial etiology and both genetic and environmental factors contribute to the risk of the disease [10]. Pathological hallmarks of this neuro-inflammatory disease are immune cell infiltration into the central nervous system (CNS) parenchyma, demyelination, and neurodegeneration [28]. Some treatment options are currently available for the early relapsing-remitting disease stage of MS which act mostly through modulation of the immune system. However, only a small number of therapies exist for the chronic progressive disease stage in which demyelination and neurodegeneration are pathological hallmarks [25]. Therefore, finding therapies that potentially restore the myelin sheath, re-establish efficient axonal conduction, and protect axons from secondary neurodegeneration has high priority in neurological research [8].

A recent study has re-invigorated interest in insulin-like growth factor 1 (IGF-1) as a potential therapy for neuro-inflammation [1]. Whereas IGF-1 was shown to be a potent promotor of developmental myelin formation [33], its potential to enhance remyelination remained controversial [6,27,32]. The functionally and structurally related hormone insulin could yet be another approach to boost remyelination. It shows some crossreactivity at the level of recep-

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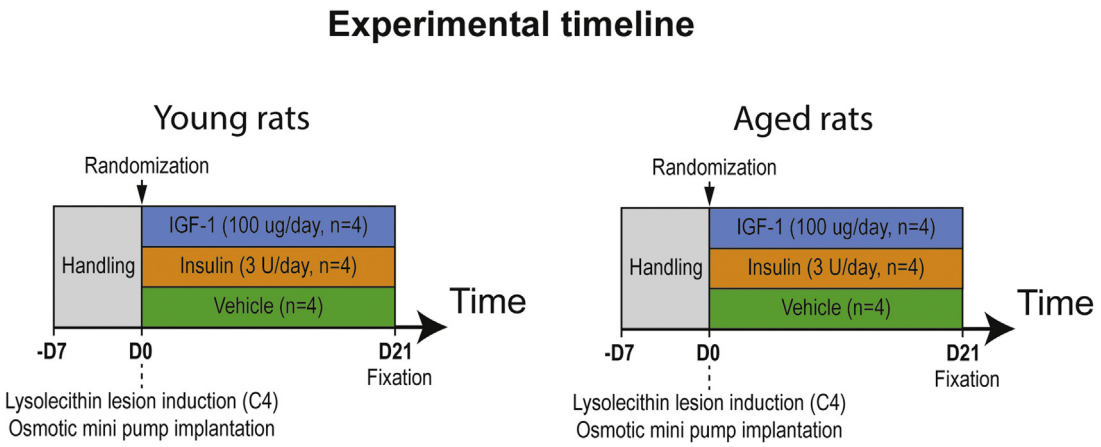

Fig. 1. Experiment timeline. After baseline measurement of food intake and blood glucose levels, both young and aged rats were subjected to a lysolecithin injection (Day 0, 2 μl, 1% lysolecithin) causing local demyelination and subsequent remyelination in the dorsal funiculus at spinal level C4. Additionally, rats were implanted with a subcutaneous osmotic minipump connected to an intrathecal catheter placed in proximity to the lesion. This minipump continuously delivered either IGF-1 (100 μg/day), insulin (3 U/day), or control solution. At day 21, rats were sacrificed and remyelination was assessed. Food and water intake as well as blood glucose was measured on daily basis.

tors, shares some signaling pathways with IGF-1 [21,30] and has been shown to enhance remyelination *in vitro* [16,29].

Here, we used lysolecithin injections into the spinal cord dorsal funiculi as a model of local demyelination, and compared the effects of chronic intrathecal infusion of IGF-1 or insulin on myelin repair in young and aged rats. Aged rats show weak innate remyelination and are therefore a good model for chronic demyelination, similar to the situation in progressive MS [24].

2. Materials and methods

2.1. Animals

Young (12–14 weeks, 200–300 g) and aged (12 months, 300–400 g) female Long Evans rats were used for all experiments (LE, strain code 006, Charles River, Italy). Rats were housed in a specific-pathogen-free (SPF) animal facility in groups of 3–4 rats under a constant 12 h light/dark cycle (light from 06:00 a.m. to 06:00 p.m.) with standard rodent chow and water ad libitum. All animal procedures and protocols were approved by the Veterinary Office of the Canton of Zurich, Switzerland.

2.2. Lysolecithin injections

Lysolecithin injections in the cervical spinal cord were performed as previously described [2]: briefly, rats were deeply anesthetized under fentanyl (0.005 mg/kg), medetomidine (0.15 mg/kg), and midazolam (2 mg/kg). The cervical spinal cord at spinal level C4 was subsequently exposed. Using a 35 gauge Hamilton syringe (World Precision Instruments, Sarasota), 2 μl of a sterile 1% lysolecithin solution (Sigma, St. Louis) in phosphate-buffered saline (PBS) was slowly injected (10 nl/s) in the midline 400 μm deep targeting the dorsal funiculi. At the end of the injection, the injection cannula remained in position for 2 min until withdrawal. The injection position in the spinal cord was marked by applying a droplet of Monestral blue after the injection.

2.3. Intrathecal insulin and IGF-1 treatment

To ensure sufficient local bioavailability of IGF-1 and insulin, the compounds were applied intrathecally *via* a catheter inserted to the subdural space of the cisterna magna. This catheter was pushed over the spinal cord in caudal direction so that the catheter tip was in proximity to the lysolecithin lesion at spinal level C4. It was connected to a subcutaneous osmotic minipump (Alzet, model 2ML2, Germany), subcutaneously implanted on the back of the rats as previously described [19]. An experimental timeline is depicted in Fig. 1.

The pump supplied the intrathecal space constantly with either 100 μg/day IGF-1 (Peprotech, USA), 3 units (U)/day (=104 μg/day) insulin (Actrapid, HM Penfill), or vehicle solution over a total treatment period of 3 weeks. IGF-1 and insulin were diluted in the vehicle solution consisting of 10 mM acetic acid, 0.1 M NaCl, and 0.1% Tween, pH 5.4 [6]. These concentrations of IGF-1 and insulin have equivalent molar concentrations. The catheters were flushed before implantation with the corresponding treatment solution in order to avoid sticking of the compounds to the catheter walls.

2.4. In vivo measurements

Since centrally applied insulin can lead to a reduction of food and water intake *via* the hypothalamus [4,12,13], we monitored weight as well as food and water intake during night and day by weighing water bottles and chow twice daily (08:00 a.m. and 08:00 p.m.). To measure potential decreases in blood glucose levels by insulin reaching the periphery from the intrathecal space, blood glucose was measured once a day by sampling a small droplet of blood taken from the tip of the rat tail. Blood sugar was measured by blood glucose test sticks and the corresponding console (Freestyle Lite, Abbott, USA).

2.5. Analysis of remyelination

Series of semithin sections stained with toluidine blue were taken at the level of the lesion center (defined in the intact spinal cord under a stereomicroscope), and approximately 1 mm caudally and 1 mm rostrally of the lesion center and analysed by light microscopy at 600×. The amount of disproportionally thin myelin compared to the axon diameter (representing remyelinated axons [2]) was quantitated in the images of these lesions within the dorsal funiculus using imageJ (NIH, USA). The sensory tracts (cuneate and gracile fascicles) of the dorsal funiculus consist mainly of large diameter axons allowing unequivocal identification of remyelinated axons. These counts were normalized to the lesion area and expressed as remyelinated axons per 1000 μm^2. One rat of the control group of aged rats had to be excluded from the analysis due